extent and types of cytotoxic T-cells deployed against *T. parva.* This information will lead to a better understanding of immune responses against the parasite.

Reaping the harvest of molecular biology

The polymerase chain reaction is certain to continue to play a major role in ILRAD's biological research—molecular and otherwise. It is startling that a technique still in its infancy has become essential to so many of the institute's research projects. As scientists learn more subtle and powerful ways to use the technique, its contributions to biomedical and veterinary science will also proliferate, perhaps starting an acceleration in research progress not unlike the exponential power of the chain reaction itself.

Studies of a trypanosome enzyme that may prove useful as an antigen (Ph.D.Thesis)

The parasite *Trypanosoma congolense* causes the disease trypanosomiasis. Infection begins when trypanosomes are injected into the blood of a mammal by a tsetse fly as it feeds on the animal. In the animal host and the tsetse fly vector, the parasites pass through several developmental stages in their life cycle.

As part of ILRAD's research into improved methods of controlling trypanosomiasis, scientists are investigating parasite components that might be attacked without harming cells of the host animal. In this area, biochemical studies were conducted on the activity of proteolytic enzymes of the parasite. These enzymes break down protein molecules into smaller fragments. The studies were designed to determine where these enzymes are located within the parasites and whether proteolytic enzymes occur only in particular stages of the parasite's life cycle, in which case they are known as 'developmentally regulated', or whether they occur in all stages.

An experiment was conducted to examine the activity of proteolytic enzymes in different developmental forms of *T. congolense.* The results indicated that in bloodstream and metacyclic forms of the parasite, enzyme activity occurs inside the lysosome, a cell structure rich in enzymes that functions in intracellular digestion; in epimastigote insect forms, however, enzyme activity occurs outside the lysosome, in the cytosol of the cell (the soluble portion of the cytoplasm).

A cysteine protease (so-called because the enzyme contains the amino acid cysteine in its active site) was purified from the lysosome-like organelles of bloodstream forms of *T. congolense* and from suspensions of ruptured parasites. Rabbit antibody to the cysteine protease was prepared and used to determine where the protease is located in the parasite. The polyclonal antibody raised against the purified enzyme was labelled with markers. Viewed in the electron microscope, the markers appeared in parasite structures resembling lysosomes. It was also found in the flagellar pocket, through which the parasite engulfs nutrients, and in other structures that are part of the parasite's endocytotic (nutrient uptake) network.

The presence of the cysteine protease within the flagellar pocket suggests that direct contact is possible between the enzyme and host animal and, furthermore, may result in pathologic degradation of important host proteins. Interestingly, collaborative studies with Dr. Edith Authie (ILRAD) suggest that trypanotolerant N'Dama cattle make antibodies against this enzyme during the course of infection, whereas cattle that are susceptible to the disease do not.

Fragments of .the trypanosome gene that codes for the cysteine protease were isolated, cloned and partially sequenced. The full gene was found to be at least 1.6 kilobases long. Messenger RNA was isolated from all four life cycle stages of the

parasite. The cloned cysteine protease gene hybridized to a 2.1-kilobase messenger RNA from metacyclic and bloodstream forms but not from procyclic and epimastigote forms. Furthermore, the hybridization signal was 4 to 5 times stronger in the metacyclic forms than in the bloodstream forms. This suggests that more of this genetic message is expressed in metacyclic forms and that transcription of the message is turned on in metacyclic forms, turned down in bloodstream forms and turned off in other life cycle stages. These results indicate that proteolytic enzymes of *T. congolense* are developmentally regulated and that the enzymes play an important role in the viability of the parasites in their mammalian hosts.

Zeres Mbawa Nkhungulu,
Zambian ILRAD Research Fellow.
Ph.D. thesis abstract submitted in
1992 to the Department of Biomedical Sciences,
Samora Machel School of Veterinary Medicine,
University of Zambia,
Lusaka, Zambia.

Ph.D. Supervisor: J. Lonsdale-Eccles.

The main article in this issue is based on reports of recent ILRAD research by Stephen Kemp (bovine genome mapping), Richard Bishop (East Coast fever epidemiology), Albert Bensaid (T-cell receptors) and Alan Teale (diagnosis of trypanosomiasis). The latter report was adapted from a paper published in a proceedings entitled Biotechnology in Livestock in Developing Countries, ed. by A.G. Hunter, Edinburgh: Centre for Tropical Veterinary Medicine, 1991, pp. 272–285.

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ILRAD was founded in 1973 to conduct research into better ways of controlling livestock diseases. The current primary goal of the Laboratory is to develop safe, effective and economical methods to control two parasitic diseases that severely constrain animal production in Africa: trypanosomiasis, transmitted to animals by the bite of a tsetse fly, and East Coast fever, a virulent form of theileriosis, transmitted to cattle by ticks. An international staff of about 50 scientists conducts basic research, much of it aimed at the development of vaccines, in the fields of biochemistry, cell biology, electron microscopy, epidemiology, genetics, immunology, molecular biology, pathology, parasitology and the socio-economics of animal disease control.

ILRAD is one of 17 international agricultural research centres sponsored by the Consultative Group on International Agricultural Research (CGIAR). The secretariat of the CGIAR is located in the World Bank headquarters, in Washington, D.C. The CGIAR is an informal umbrella organization of 40 national governments, international organizations and private foundations that together provide about US$300 million annually to the 17 centres for research, training and advisory services The CGIAR aims to help farmers in developing countries increase their production of staple food crops, livestock, fish and trees in ways that improve the nutrition and well-being of low-income peoples and the management of natural

resources.